Vaccinating against bone destruction

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A preliminary study in mice suggests that a therapeutic vaccine against the receptor-activator of NF-κB ligand (RANKL) could represent a novel approach for the treatment of osteoporosis, rheumatoid arthritis (RA) and other diseases associated with increased osteoclast activity and bone loss. Results presented at the *23rd Annual Meeting of the American Society of Bone and Mineral Research* (ASBMR; 12–16 October 2001, Phoenix, AZ, USA) showed that a RANKL vaccine developed jointly by researchers at the University of Tokyo (Tokyo, Japan) and Pharmexa AS (Hørsholm, Denmark) could protect mice from bone loss induced by removal of both ovaries (a model of post-menopausal osteoporosis) and also reduced inflammation and bone destruction in a mouse model of RA[1,2].

Bone-related disorders can take on many forms: post-menopausal osteoporosis, glucocorticoid-induced osteoporosis, male osteoporosis, RA, periodontal disease and bone metastasis. Although the forms of disease vary, the underlying mechanisms are the same: an imbalance in bone resorption and bone formation in favour of resorption leads to reduced bone mineral-density, reduced bone strength and subsequently increased skeletal fractures, which impact heavily on the quality of life causing deformity, increased mortality and excruciating pain.

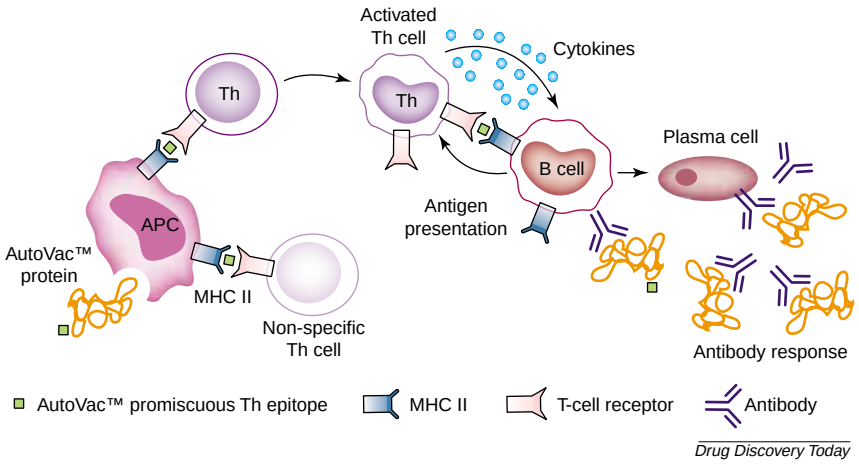

Figure 1. AutoVac™ can bypass immunological tolerance by stimulating B and T lymphocyte activation. The AutoVac recombinant DNA or protein is taken up and processed by antigen presenting cells (APCs), which present the derived peptides in association with major histocompatibility complex (MHC) class II molecules. T helper (Th) cells recognize these promiscuous epitopes presented by the APCs, and become activated to produce cytokines. Although peptides from the self-portion of the Autovac antigen are also presented on the MHC II, Th cells that recognize these self-peptides are removed from the repertoire during tolerance and are not present. The cytokines produced by the activated Th cells stimulate B cells that have internalized the vaccine to differentiate into antibody-secreting plasma cells, which secrete antibodies against the AutoVac protein. Simultaneously, B cells that recognize the antigen from the vaccine internalize the AutoVac protein via B-cell antigen receptors and process and present peptides in association with MHC class II molecules to Th cells. This interaction further stimulates T-cell help for the B cell.

The role of RANKL

RANKL is a cytokine that acts with osteoprotegerin (OPG) as an essential regulator of osteoclast biology. 'Osteoclasts represent a differentiated cell type derived from the macrophagic/monocytic cell line that is extremely good at chewing up bone,' explains Lorenz Hofbauer (Philips-Universität, Marburg, Germany). In immune-mediated diseases (RA, peridontitis) or certain types of cancers (breast cancer, multiple myeloma), abnormal immune cells or cancer cells employ this machinery to achieve their detrimental action. 'The consequences include joint destruction and crippling (RA), alveolar bone loss and tooth loss (peridontitis), and fractures without appropriate trauma resulting from osteolytic metastases' says Hofbauer, whose recent review gives a good overview of RANKL and its role in bone physiology[3]. 'Overexpression of RANKL can be demonstrated in patients suffering from bone-related disorders such as osteoporosis and RA, suggesting that RANKL is a valid therapeutic target,' says Marc Hertz, Senior Researcher at Pharmexa.

Overcoming immunological tolerance

Under normal circumstances, the immune system does not respond to RANKL because it is a self-antigen and is subject to immunological tolerance. 'In recent years, the path to therapeutic vaccines has not been an easy one, but the technology now exists to enable vaccines to be developed that circumvent immunological tolerance,' explains Hertz[4]. The AutoVac™ technology activates T helper (Th) cells, which then bypass immunological tolerance and induce robust

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immune responses. 'This is achieved in the vaccine by incorporating a ''promiscuous'' T-helper (Th) epitope into the self-protein,' explains Hertz.

This epitope forces the immune system to respond, even when the antigen in the vaccine is normally recognized as self. 'The promiscuous Th epitope stimulates the Th cells that, in turn, stimulate B lymphocytes to produce antibodies capable of neutralizing the self-protein,' adds Hertz (Fig. 1). Importantly, the immune response is dependent on the AutoVac antigen to maintain activation of the Th cells. Furthermore, the endogenous protein is not capable of boosting the immune response, thus avoiding an autoimmune state. The technology can also be applied to diseases associated with the pathological expression of other proteins, and currently a TNFα and a Her-2 AutoVac are being tested in Phase I/II clinical trials.

In vitro studies

The RANKL vaccine used in the current study was developed by modifying the soluble TNF-like domain of murine RANKL (amino acids 158–316) to incorporate a promiscuous Th epitope. 'We have shown that a rodent RANKL AutoVac can induce antibodies in a mouse that crossreact and neutralize native non-modified RANKL and prevent osteoclast formation *in vitro* and *in vivo*,' said Hertz. 'The most striking results were obtained in a mouse model of RA, in which the mouse develops RA-like symptoms at two months of age. Vaccination using RANKL AutoVac after the onset of disease not only prevented the formation of osteoclasts by 60% and the subsequent bone destruction by 80%, but also reduced synovial inflammation by 40%,' he continued.

Hofbauer thinks that 'Vaccination against RANKL is a promising, innovative approach, provided that an effective titre of antibodies is sustained over a long period of time. An important caveat, he adds, is that RANKL blockade could alter normal immune functions because RANKL is an important stimulator of dendritic cells, and thus, a crucial regulator of both cellular and humoral immune responses. 'Long-term follow-up in these mice will show whether RANKL blockade is associated with increased susceptibility to opportunistic infections,' he stresses.

Future plans

Co-author, Sakae Tanaka (University of Tokyo, Japan) confirms that the collaborative team is planning to examine the effect of the vaccine in various animal models that exhibit pathological bone resorption, such as a rat ovariectomy model, metastatic bone-tumour models and other arthritis models. 'Afterwards, we hope to move forward to clinical trials of the RANKL-AutoVac not only in Europe, but also in Japan,' he says. Hertz hopes that the vaccine will enter Phase I/II studies by 2003 for bone metastasis patients. 'Bone metastases are a major complication in both breast cancer and multiple myeloma, causing bone destruction and severe pain,' he comments. 'I believe the RANKL vaccination is a powerful approach to any kind of pathological bone resorption. Metastatic bone tumours, RA with severe bone destruction and osteoporosis would all be good targets of RANKL-AutoVac,' adds Tanaka.

References

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